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Integrins and Integrin-related Proteins in Cardiac Fibrosis

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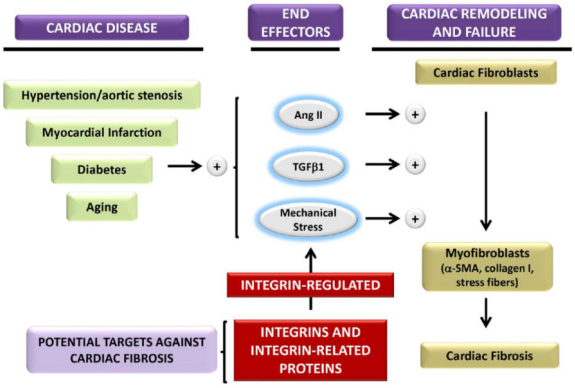
Abstract

Cardiac fibrosis is one of the major components of the healing mechanism following any injury of the heart and as such may contribute to both systolic and diastolic dysfunction in a range of pathophysiologic conditions. Canonically, it can occur as part of the remodeling process that occurs following myocardial infarction or that follows as a response to pressure overload. Integrins are cell surface receptors which act in both cellular adhesion and signaling. Most importantly, in the context of the continuously contracting myocardium, they are recognized as mechanotransducers. They have been implicated in the development of fibrosis in several organs, including the heart. This review will focus on the involvement of integrins and integrin-related proteins, in cardiac fibrosis, outlining the roles of these proteins in the fibrotic responses in specific cardiac pathologies, discuss some of the common end effectors (Angiotensin II, transforming growth factor beta 1 and mechanical stress) through which integrins function and finally discuss how manipulation of this set of proteins may lead to new treatments which could prove useful to alter the deleterious effects of cardiac fibrosis.

Abstract



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Keywords

Fibrosis; integrins; myocardium; transforming growth factor beta; angiotensin; mechanical stress

1. Introduction

Heart failure is a major cause of morbidity and mortality in the western world with limited numbers of therapeutics that impact the primary disease process [1]. Both heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF) display a range of physiological and morphological changes, including fibrosis of the myocardium. Fibrosis is the excessive deposition of extracellular matrix (ECM) proteins into tissues, leading to scar formation, disruption of normal tissue architecture and potentially to organ failure [2]. In particular, cardiac fibrosis is one of the major components of the healing mechanism following any injury of the heart and as such may contribute to both systolic and diastolic dysfunction in a range of pathophysiologic conditions [3]. Canonically, it can occur as part of the remodeling process that occurs following myocardial infarction (MI) or that follows as a response to pressure overload (PO). It can even be currently tracked non-invasively in man, through use of gadolinium-based magnetic resonance imaging [4].

Multiple organs in the body can be affected by fibrosis, and a large effort has been focused on studying the fibrotic response that occurs in diseases of lung, kidney, liver and skin. Although the triggering events which lead to the fibrotic disorders in these non-cardiac organs could be quite different, the fundamental processes that drive fibrosis are likely to be common in most tissues throughout the body, including the heart. Importantly, disorders that lead to fibrosis can share the complex interplay between inflammatory, epithelial, myofibroblast and ECM responses [5–7]. Myofibroblasts are a main cell type that fuel fibrosis and have combined characteristics of fibroblasts and smooth muscle cells. As such they have a contractile phenotype and contribute significantly to the formation of scarring by secreting ECM components [8]. The origin of myofibroblasts in different organs has been intensely studied, with potential sources suggested to include resident fibroblasts, circulating progenitors (fibrocytes) and also potentially cells that arise by the process of epithelial – mesenchymal transition (EMT) [9, 10].

Integrins are cell surface receptors which act in both cellular adhesion and signaling. Most importantly, in the context of the continuously contracting myocardium, they are recognized as mechanotransducers [11–13]. They have been implicated in the development of fibrosis in several organs, including the heart [14]. In addition to their direct effects on cellular proliferation, migration and survival, mediated by their binding to ECM proteins, integrins can potentiate signals from soluble growth factors such as transforming growth factor β1 (TGFβ), and act as a receptors for matricellular proteins [15]. All of these properties allow integrins and proteins which interact with them, to play essential roles in the fibrotic process.

This review will focus on the involvement of integrins and integrin-related proteins, in cardiac fibrosis. While fibrosis can occur as a component of a wide variety of myocardial diseases, this review will focus on examples that occur following MI and as a result of

hemodynamic overload, aging and diabetes. Here we will provide information on how integrins are affected and involved in cardiac fibrosis. In the first part we will introduce basic information about integrins and integrin-related proteins. Then, we will outline the roles of these proteins in the fibrotic responses of specific cardiac pathologies, discuss some of the common end effectors through which integrins function and finally discuss how manipulation of this set of proteins may lead to new treatments which could prove useful to alter the deleterious effects of cardiac fibrosis.

2. Integrins and integrin-related proteins

There have been several recent excellent reviews written about the structure, function, expression and extensive animal modeling studies of integrins and integrin-related proteins. These include ones relevant to the heart, by our own group and others [11, 12]. Given this, here we will only provide a brief background on these proteins prior to our discussion of their role in the fibrotic process.

2.1 Integrins

Integrins are transmembrane receptors which act as bridges for cell-ECM connections and in some instances, cell-cell interactions. Thus one of their prime functions is to couple the ECM outside cells, to the cytoskeleton inside the cell. Integrin receptors are obligate heterodimers, composed of two different chains, termed the α and β subunits. In mammals there are 18 α and 8 β subunits, which combine to make up 24 different integrin combinations [16]. The integrin subunits can vary from 90 – 160 kDa and generally consist of a large extracellular domain, a single transmembrane spanning domain, and a short cytoplasmic tail [17]. The cytoplasmic domain of many of the β subunits is highly homologous, while the α subunit sequences are significantly more diverse. It is through the cytoplasmic tail, dominantly of β subunits, that the integrins bind both cytoskeletal linkers and also signal. (Figure 1)

Cell attachment to the ECM is a basic requirement to build a multicellular organism. Integrins are part of the cell adhesion complexes, which along with many cytoplasmic structural and signaling proteins, such as talin (Tln), vinculin (Vcl), paxillin (Pax), focal adhesion kinase (FAK), α-actin and integrin-linked kinase (ILK), serve to link two networks across the plasma membrane: the ECM and the intracellular actin filamentous system [18]. Thus integrins and their associated proteins, are not simply hooks in a cellular meshwork, but provide the cell with critical inputs about the nature of its surroundings. Although integrins do not possess their own enzymatic activity, they are potent bidirectional signaling receptors, playing an important role in cell signaling [19, 20]. When triggered by ligands, integrins can influence a host of downstream biochemical pathways in the cell interior, a process commonly termed **outside-in signaling**. This type of signaling may allow sensing of both chemical composition and mechanical status of the ECM outside the cell. Then, depending on the integrin's regulatory impact, the cell can experience growth, proliferation, division, differentiation or other means of remodeling. In addition, the control of integrin function occurs via regulatory signals that originate within the cell cytoplasm and are then transmitted to the external ligand-binding domain of the receptor. This concept is known as

inside-out signaling. It can increase both binding of integrin to ligand (ECM) and lead to clustering of multiple integrins in close spacing within the cell membrane.

The variety of integrin receptors expressed on a particular cell type can be unique. Further, expression of integrins may not only be restricted to a particular cell type, but can vary depending on developmental stage or pathological state. In addition, functional complexity of integrins also occurs since a single integrin receptor can bind to one or several ligands, and in addition, a single ligand can be bound by several integrin heterodimers. For example, in the cardiac myocyte (CM), the integrin heterodimers most highly expressed are α1β1, α5β1, and α7β1, which are predominantly collagen, fibronectin, and laminin-binding receptors, respectively. In addition, α6, α9, and α10 are also detected in myocytes. β1 is the dominant CM β integrin subunit, but β3 and β5 subunit function have also been studied [21–23]. In contrast, cardiac fibroblasts (CFs) express α1β1, α2β1, α3β1, α4β1, α5β1, α6β1, as well as αvβ1, αvβ3 and αvβ5. These integrin pairs play critical roles in cardiac remodeling via ECM-integrin interaction. Endothelial cells (EC) express α1β1, α2β1, α5β1 and αvβ3 integrins. As will be relevant to later discussion, β2 integrins are confined to leukocytes, being expressed on cells such as macrophages and neutrophils.

Matrix metalloproteinases (MMPs), a large family of calcium-dependent zinc-containing endopeptidases can mediate degradation of ECM components during various physiological and pathological processes. Some ECM components, through interaction with integrin receptor and modulation of downstream signaling, are capable of regulating expression and activity of several MMPs. One example is that α4β1, α5β1 as well as αvβ3 integrins can mediate expression and activity of MMPs and their effector responses, in different cellular systems. Damsky's group found that when FN is bound to α4β1 and α5β1 integrins, MMP expression in rabbit synovial fibroblasts is regulated [24]. This occurred when the fibroblasts were cultured on surfaces coated with the central RGD-containing region of FN (120FN) as well as when the cells were incubated with anti-α5β1 or anti-α4 antibodies. Brooks and colleagues later found that MMP-2 was localized in a proteolytically-active form on the surface of invasive cells when they bound directly to αvβ3 integrin[25].

Although integrins can alter the expression and the localization of MMPs, MMPs can also cleave integrins and affect the integrin signaling pathway. For example, MMP-2 impairs β1 integrin-mediated survival signals produced by activation of focal adhesion kinase (FAK) to protect β –adrenergic receptor-stimulated apoptosis of adult CMs. Overexpression of β1 integrins also inhibited apoptosis induced by purified, active MMP-2, in adult CMs [26]. Recently, MMP-9 was found to cause shedding of the β2 integrin subunit (CD18) from macrophages, which suggests that MMP-9 can play an important role in β2 integrin post-translational modification [27]. Further, other studies found that MMP-2 is up-regulated in invasive colorectal tumors and with this, caused shedding of β1 integrin followed by subsequent integrin degradation. This lead to decreased adhesion and enhanced cell motility [28]. MMP mediated-shedding of integrins is a posttranslational modification which can alter integrin activation state, as well as integrin receptor internalization and degradation.

2.2 Integrin-Related Proteins

Integrins do not possess their own enzymatic or actin-binding activity. Therefore, various adaptor proteins that bind to the cytoplasmic tails of α and β subunits are required to mediate structural or scaffolding properties, and produce catalytic activity (i.e. outside-in signaling), or activate integrins to effect ECM binding (inside-out signaling). Since some of these proteins crucial for integrin function have not been investigated in the fibrotic process, we will only introduce a few of these proteins below.

Talin (Tln) is a ubiquitously expressed, cytosolic protein that is found in high concentrations in focal adhesions (FA). It is a large protein (270kDa) which links integrins to the actin cytoskeleton either directly or indirectly, by interacting with vinculin (below) and α–actinin [29]. Tln has been shown to be an essential protein for force generation and mechanotransduction [30]. In addition to its structural role, Tln is essential for integrin activation [31]. In vertebrates, there are 2 Tln isoforms, Tln1 and Tln2 [29].

Integrin Linked Kinase (ILK) is a 51 kDa serine/threonine protein kinase that interacts with β1 and β3 integrin cytoplasmic domains, linking integrins to the actin cytoskeleton and mediating integrin signaling in diverse types of cells. ILK is suggested to elicit its biologic activity through its role as a scaffolding protein, binding proteins such as PINCH and Parvin, as opposed to a role as a kinase protein [32].

Focal adhesion kinase (FAK) is a 125kDa ubiquitously expressed non-receptor tyrosine kinase that plays a major role in integrin-mediated signal transduction. FAK can be activated by either ECM (through integrins) or by growth factors, regulating multiple signaling pathway outputs [33]. The C-terminal domain of FAK promotes its colocalization with integrins through its association with integrin-associated proteins such as Pax and Tln [34]. In vivo and in vitro studies showed that FAK is rapidly activated by mechanical stress [35–37]. Proline-rich tyrosine kinase-2 (Pyk2) is a tyrosine kinase related to FAK that shares a similar domain structure and has common phosphorylation sites [38].

Kindlins are 78kDa cytosolic proteins that directly interact with the cytoplasmic tail of β integrin and are required for the correct assembly of FAs. They act as crucial co-activators of integrins. There are 3 subtype kindlins: kindlin-1, -2, and -3, that have varied expression patterns. Kindlin-1 and kindlin-2 are widely expressed in murine and human tissues, while kindlin-3 is restricted to hematopoietic tissues. There are 4 kindlin-binding proteins: ILK, migfilin, β1 integrin, and β3 integrin [39].

3. Integrins and Integrin-related proteins in cardiac fibrosis

Cardiac fibrosis, characterized by the excessive production and deposition of scar tissue, is often a result of chronic conditions such as hypertension and diabetes, or acute conditions such as MI or myocarditis. It also occurs with normal aging. There are different types of fibrosis in heart.

With death of CMs as occurs after MI, the regions of the heart from which the myocytes are lost undergoes fibrotic change termed “replacement fibrosis.” With stress related remodeling

of cardiac chambers as can occur with PO states (e.g. with hypertension or valvular stenosis,) or in the remote regions following MI, “reactive fibrosis” occurs in an interstitial or perivascular pattern. All of these types of fibrosis may lead to systolic and / or diastolic decrements in function, and further, may even predispose to arrhythmias given the loss of myocyte-myocyte connections, and potential influence of non-myocytes (e.g. fibroblasts or other cells) as they interface with myocytes [40].

Importantly, a cell death mechanism termed anoikis must be considered in the context of how integrins may be related to fibrosis. Anoikis is defined as programmed cell death induced by the loss of cell-matrix interactions [41, 42]. Thus integrins are central to this process. Anoikis may play a physiological role by regulating cell homeostasis in developing or mature tissues. However, anoikis can also be involved in pathological processes. While anoikis has been principally linked to a role in cancer, it may also serve important roles in the cardiovascular system. For instance it likely is a means to produce CM cell loss in the context of multiple myocardial stressors [43]. Lorell and colleagues found that the localization of β1 integrin was increased on the cell surface of CMs, and in the ECM surrounding CMs, during the early stage of heart failure, as perhaps integrins were shed from the cells [44]. Loss of CMs occurred soon after left ventricular pressure overload, and with this anoikis was indicated to contribute to the progression towards heart failure. Since pressure loading produced abnormal myocyte-ECM anchorage, and subsequent anoikis mediated-cell death, replacement fibrosis could ensue. Similar results could occur with other cardiovascular pathologies. Having the above as background, we will now focus on the behavior and role that integrins and associated proteins play in the fibrotic process in some key cardiac pathologies.

3.1 Cardiac fibrosis after myocardial infarction

Cardiac remodeling and particularly fibrosis, both in the post-MI infarcted and non-infarcted regions, is recognized to be a major determinant of subsequent development of impaired ventricular function [45, 46]. After MI, both replacement and interstitial fibrosis occur and are determined by the extent of myofibroblast proliferation, and also by its associated collagen deposition. Wound healing after MI entails a complex cascade of events that involves interplay between several different cell types, including inflammatory cells, endothelial cells (EC), fibroblasts and myofibroblasts [47]. Infarct healing can be divided into three distinct, but overlapping phases in man: the inflammatory phase (from infarct onset to 2 days post-MI), the proliferative phase (from 3 – 4 days to 2 weeks after infarction), and the maturation phase (from 2 weeks after MI, onward) – see below [47]. In response to post-MI cell death, intracellular contents are released from the necrotic cells and activate innate immune mechanisms, initiating an intense, but transient inflammatory response. As the infarct zone is cleared of dead cells, the inflammatory response is suppressed and infiltration of CF and EC into the infarct zone occurs. This marks the beginning of the proliferation phase. During this phase, CFs undergo dramatic phenotypic and functional changes showing high proliferative and migratory activity, acquisition of the myofibroblast phenotype with α-smooth muscle actin expression (α-SMA), and augmented matrix synthetic capacity (collagens I and III), all of which is important to maintain the structural integrity of the infarct area. TGFβ signaling, mechanical stress and specialized

matrix proteins such as the ED-A isoform of cellular fibronectin (ED-A FN), are some of the main promoters of CF to myofibroblast transdifferentation [48]. ED-A is one type of fibronectin subunit that is expressed commonly during wound healing and in areas of fibrotic change. Each FN subunit is formed from a series of repeating, homologous modules, and contains binding sites for cell surface receptors such as integrins, as well as other ECM components. FN polymorphisms follow from the alternative splicing of the type III segments and these variants are termed ED-A, ED-B, and IIICS [49]. Other factors that play important roles during this phase are angiotensin II (Ang II) and the matricellular proteins thrombospondin-1 (TSP-1), osteopontin (OPN), periostin and tenascin-C [48].

As the infarcted zone is filled with matrix, cellular proliferation is inhibited and the maturation phase begins. As the infarct matures, matrix cross-linking results in the formation of a dense, collagen-based scar. Collagen deposition also occurs in the un-infarcted, remote myocardial region, predominantly in the interstitium, where these deposits clearly contribute to ventricular stiffness, arrhythmogenesis and dysfunction [50]. Neurohumoral factors, such as Ang II and TGFβ1, are preferentially expressed at the infarct border. There they traverse the common interstitial space and enhance collagen deposition at sites distant from the MI, contributing to the remote interstitial fibrosis. Some studies also suggest that mechanical alteration in the infarcted myocardium leads to persistent myofibroblast expansion and excessive ECM production, due to the failure of the apoptotic mechanism of these cells, favoring remote zone fibrosis [51].

Undoubtedly, since integrins serve at the interface between cells and ECM, they are involved in the different healing / remodeling phases after MI. Initial work has been performed to evaluate the changes in several α integrin subunits after MI in the rat [52]. In hearts without infarction, no expression of α1 integrin, moderate expression of the α3 integrin and only slight expression of the α5 subunit were observed in myocardium. In the first week after MI, the α1 subunit, collagen and fibronectin were increased only in the peri-infarcted area, while the α5 subunit was increased both in peri-infarcted and non-infarcted areas. At day 42, the expression of the α1 subunit and collagen were still increased, although the α5 subunit and fibronectin were decreased. The expression of the α3 subunit was not altered throughout the experimental period. Thus α1 integrin remained increased during a significant duration after MI, in the peri-infarcted and non-infarcted areas; α5 integrin expression levels increased during the healing process and decreased during the remodeling process. This data suggests that different α integrin subunits may play varied roles during the MI process.

β integrins may also play an important coordinating role in ECM synthesis and remodeling in the heart, as was demonstrated previously in skin and lung injury [53–55]. Liu's group studied the role of β integrins after MI using rat and mouse model systems [56]. Both β1 and β3 integrins had low expression basally, but increased at the infarct zone by day 3 post-MI, peaking at day 7 post-MI, then gradually declined thereafter and returned towards baseline. In line with the cell-type specific expression of β1 integrin isoforms, the β1A isoform was found primarily expressed on CF and inflammatory cells, while the β1D isoform was expressed on CMs. β3 integrin was detected principally on ECs and smooth muscle cells in the peri-infarct vessels [56]. The temporal expression and spatial localization of these various integrin subunits suggests they play an important role in healing and remodeling

processes after MI. In other studies, Singh's group used β1 integrin heterozygous knockout (hKO) mice and studied them one month after MI [57]. MI increased β1 expression in both KO and control groups, with the increase mainly observed in the peri-infarct and non-infarcted areas. However, the increase was lower in hKO, that had one β1 integrin allele deleted, and the hKO group displayed worsened cardiac function than the WT controls, after MI. Increased apoptosis and cardiac fibrosis in the hKO was also found after MI. Together this data suggests that β1 integrins are crucial in post-MI remodeling [57].

ILK expression was also found to be increased following MI, in the infarct area [58]. Forced increase of ILK expression, using gene therapy, has been shown to improve cardiac remodeling in rats after MI. Recombinant adenoviral-ILK was delivered during coronary ligation induced MI, in the rat. At the 4-week follow-up study, ILK treated animals showed improved cardiac function, along with decreased: a) infarct size, b) interstitial fibrosis and c) CM apoptosis, as compared to the control group [59, 60]. Additionally, ILK- overexpression in mesenchymal stem cells injected into porcine peri-infarct myocardium 7 days post-MI, preserved cardiac function and myocardial perfusion, reduced fibrosis, increased CM proliferation, and enhanced angiogenesis [61]. These studies suggest ILK overexpression may preserve cardiac function and reduce cardiac fibrosis post-MI.

Although activated myofibroblasts are the main effector cells in the fibrotic heart, monocytes / macrophages, lymphocytes, and mast cells also contribute to the fibrotic response. The mechanisms how these immune cells work on healing and remodeling post-MI has been recently extensively reviewed and will not be discussed here [62–67]. Yet, different immune cells express varied integrins. In leukocytes, β2 as well as α4β1 and α4β7 (LPAM-1) integrins play roles in cellular recruitment and in inflammatory disorders. The β2-integrins consist of a common β subunit (CD18) that associates with 4 different α subunits. They comprise the αLβ2-integrin (lymphocyte function-associated antigen 1 (LFA-1); CD11a/CD18) and the αMβ2-integrin (macrophage-1 antigen (Mac-1) also designated complement receptor 3 (CR3); CD11b/CD18), which are the most crucial β2-integrins for leukocyte recruitment, as well as the αXβ2 (CD11c/CD18; p150,95; CR4) and the αDβ2 (CD11d/CD18) integrins [68]. LFA-1 is expressed by neutrophils, monocytes and lymphocytes, whereas Mac-1 is found mainly on neutrophils and monocytes, and VLA-4 is expressed on monocytes and T lymphocytes [68–71]. αXβ2 is present on macrophages and dendritic cells [72, 73], and αDβ2 is expressed on monocytes/macrophages, especially foam cells, which are macrophages found in atherosclerotic lesions [74, 75].

Since immune cells are an essential part of the healing and remodeling process, and different immune cells express varied integrin receptors, anti-integrin therapeutic approaches could be promising to reduce infarction size and adverse post-MI remodeling. Both antibody neutralization studies (anti-Mo1, anti-CD11b) [76] and genetic loss-of-function models [77], documented the crucial role of β2 integrins, in recruitment of neutrophils into the infarcted myocardium, and in modifying how adherent neutrophils transmigrate into the infarct through interactions that may involve endothelial transmembrane proteins, including platelet endothelial cell adhesion molecule (PECAM)-1, Intercellular Adhesion Molecule 1(ICAM-1), vascular-endothelial (VE)-cadherin and members of the junctional adhesion molecule family. Several independent studies in animal models have suggested marked

reduction in infarct size upon administration of anti-CD11/CD18 antibodies [76, 78, 79]. Unfortunately, three small clinical trials showed no effects of early anti-integrin approaches in reducing infarct size in human patients with MI [79–81].

Administration of the anti-α4 integrin antibody (natalizumab) was found to block macrophage trafficking to the heart in a Simian immunodeficiency virus (SIV) infection model of AIDS. With this, it decreased macrophage numbers in cardiac tissues, and decreased fibrosis in the SIV-infected group [82]. These data demonstrate a role for macrophages in the development of cardiac inflammation and fibrosis, and suggest that blocking α4 integrin, with subsequent reduction of monocyte/ macrophage traffic to the heart, can alleviate SIV/HIV and potentially be useful as a therapy with other viral-associated myocarditis and associated fibrosis.

As even further evidence of integrin-linked immune responses to cardiac fibrotic disease, are studies with CD11b/CD18 integrin knockout (KO) mice which showed reduced Ang II induced atrial fibrosis and atrial fibrillation, likely by inhibition of neutrophil infiltration during this process. In summary, modification of integrin expression / function on inflammatory cells might be a target that can reduce cardiac fibrosis.

3.2 Pressure overload and fibrosis

Fibrosis also underlies the remodeling response which occurs in the mammalian heart as it responds to hemodynamic loading provoked by PO. Arterial hypertension induces long term structural changes in the myocardium that together may act as risk factors for the evolution from a compensated state, towards heart failure. These responses include cardiac hypertrophy and cardiac fibrosis. Remodeling secondary to hypertension is attributed to mechanical stress, and to the hypertrophic, pro-inflammatory and pro-fibrotic effects of vasoactive factors such as Ang II, catecholamines, aldosterone, endothelin and TGFβ1, which are released by the cells within the stressed myocardium. In this process, fibrosis is found in spatially distinct locations - interstitial fibrosis that occurs around CMs, and perivascular fibrosis, which is detected in the vicinity of large coronary vessels. With development of fibrotic remodeling of the cardiac interstitium, increased stiffness, arrythmogenesis and diastolic dysfunction can result [83]. Fibroblasts respond to alterations in mechanical loading by enhancing their matrix-synthetic capacity and as discussed above, by transdifferentiating into myofibroblasts. Matricellular proteins also contribute to the fibrosis following PO [15].

Recent work has begun to show that integrins and their downstream kinase FAK, are affected and involved in PO mediated cardiac fibrosis [84]. For example, Burgess, et. al. studied rats subjected to treadmill exercise or hypertension. In these models, β1 integrin expression was elevated in CFs, while α1 and α2 integrin levels both decreased, but α5 integrin increased, in the exercise group and decreased in the hypertensive one [85]. These results show that CFs respond to changing environments in pathophysiological conditions by modulating integrin expression. FAK also was found to play a role in the activation of CFs in response to cyclic stretch and in the development of cardiac fibrosis provoked by PO [86]. FAK silencing by small-interfering RNA (siRNA) attenuated fibrosis, collagen content, and activity of matrix metalloproteinase-2 (MMP-2) in the overloaded left ventricle (LV) [87].

Another study showed that CF β3 integrin is critical for ECM accumulation during PO hypertrophy in mouse. β3 KO mice displayed attenuated cardiac fibrosis compared to WT controls. CFs from β3 KO mice exhibited a significant reduction in cell-matrix adhesion, cell spreading, proliferation and migration. In addition, the activation of platelet-derived growth factor (PDGF) receptor associated tyrosine kinase, and the non-receptor tyrosine kinase Pyk2, were impaired in β3 null cells following PDGF stimulation. These results indicate that β3 integrin-mediated Pyk2 signaling in CF plays a critical role in PO -induced cardiac fibrosis [88].

3.3 Cardiac fibrosis in aging

Fibrosis is also a hallmark of cardiovascular aging [89]. Development of heart disease has high prevalence in the elderly and has multifactorial causes that can summate in heart failure. Accumulation of collagen with resultant fibrosis is considered as a major contributor to reduced ventricular compliance, potentially contributing to HFpEF [90]. The increase in fibrosis not only affects the mechanical properties of the heart but has also been proposed to affect electrical properties, which may lead to arrhythmias and sudden cardiac death. Since integrins and their related protein partners mechanically couple the ECM with the intracellular cytoskeleton, it is proposed that these connections can be a component of aging related cardiac fibrosis [91].

With numerous changes occurring in the ECM of aging heart, it is reasonable to speculate that age-associated changes in integrin expression are a component of this process. Aged mice (20 months) express higher levels of collagen and fibronectin than middle aged (12 months) and younger mice (2 months). Consistent with the temporal increase in the fibronectin and collagen content within the heart, are parallel increases in the expression of integrin subunits which can bind these ECM proteins, i.e., α1 and α5 integrins. In contrast, the β1 integrin levels in old mouse hearts is significantly less than that detected in middle aged and young mice [92]. Age-associated decreases in β1 integrin was also observed in the myocardium of Wistar-Kyoto rats [93]. Likewise, adult CFs express lower levels of β1 integrins when compared to neonatal CF [94] and aged myocytes exhibit decreased levels of β1, α3, and α3β1 integrins, when compared to younger adult myocytes [95]. While little work has been performed investigating how loss of integrin subunits might affect cardiac aging (e.g. using various KO mouse models) it is interesting to note that reduction of β1 integrin in CMs also induced cardiac fibrosis [96], which suggests that maintenance of normal β1 integrin levels in CMs may be required to prevent cardiac fibrosis in the aged myocardium.

3.4 Cardiac fibrosis in diabetic cardiomyopathy

Fibrosis is a frequent complication of diabetes mellitus in many organs and tissues but the mechanism of how diabetes impacts the formation of fibrotic lesions is not well defined [97]. Diabetic cardiomyopathy is characterized by the production of a disorganized, fibrotic matrix in the absence of coronary atherosclerosis and associated ischemic damage, or hypertension. Myocardial fibrosis and collagen deposition are the primary structural changes observed in diabetic cardiomyopathy [98–101]. Cardiac fibroblasts may play a key role in the in the elaboration of fibrosis in the diabetic heart with increased proliferation causing

remodeling of collagen [102], and also by induction of a switch from a fibroblast phenotype, to that of a myofibroblast, which is both profibrotic and highly contractile [103, 104].

In a recent paper, in which streptozotocin (STZ)-treated Sprague-Dawley rats were used as a diabetes model, Talior-Volodarsky and collages found that α11 integrin transcript and protein expression were increased in isolated CFs from STZ rats vs. controls. Knock-down of α11 integrin expression with siRNA, in human fibroblasts plated on methylglyoxal-treated collagen (a glycated collagen present in the diabetic heart) blocked the increase in transcript levels of TGFβ2 (a critical end-effector in the fibrotic process, see below) and the increased protein expression of α-SMA. This result suggests that α11 integrin and TGFβ2 could mediate myofibroblast differentiation in CFs in the diabetic heart, and therefore this integrin subunit may contribute to fibrosis associated with diabetic cardiomyopathy [105].

4. End effectors of the fibrotic process

A complex interaction among a network of growth factors, cytokines and hormones are responsible for initiating and maintaining fibrotic responses in vivo. In particular, Ang II and TGFβ1 appear to work together to induce activation of resident fibroblasts, promote persistence of myofibroblasts and induce expression of a variety of ECM components, in the myocardium [106]. Recently, it has become increasingly appreciated that in addition to these factors, the cellular microenvironment also plays a critical role in cardiac fibrosis. Here we will discuss how these common effectors lead to fibrosis in most tissues, including the heart. They have been shown to be regulated by or modulated through integrins and thus are particularly relevant to the current topic.

4.1 Integrin and integrin-related proteins in Ang II induced cardiac fibrosis

Ang II plays a critical role in cardiac remodeling. This peptide promotes CM hypertrophy and interstitial fibrotic changes associated with PO hypertrophy, post-MI remodeling, and congestive heart failure. Importantly, Ang II is both expressed and activated by macrophages and myofibroblasts during remodeling after MI [107]. In CM and CF, Ang II induces the expression of TGFβ1 (see below) through the angiotensin type I receptor [108], and, in vivo, TGFβ is required for Ang II to cause both cardiac hypertrophy and fibrosis [109]. This data supports the hypothesis that Ang II is upstream of TGFβ1 in driving cardiac fibrosis. In this regard, angiotensin receptor blockers such as losartan, are effective in reducing cardiac fibrosis in both animals and humans [110, 111].

Ang II can affect integrin expression and at the same time, integrins can regulate the expression of the angiotensinogen gene. Ang II treatment of CFs in vitro has been shown to increase the expression of αvβ3 and α8β1 integrins [112–114]. A 14 day infusion of Ang II into rats increased the expression of α8β1 in the myocardium, where it was localized in vascular smooth muscle cells (VSMC) and myofibroblasts [115]. Carver et al., showed that α8β1 integrin plays a role in collagen contraction by CFs [116]. Though remaining controversial, some data show that deletion of the α8 integrin gene does not protect mice from myocardial fibrosis in a model of desoxycorticosterone-acetate (DOCA)-salt hypertension [117]. In addition, β1 integrin has been found to regulate expression of angiotensinogen in CFs following mechanical stretch. This effect is mediated by activation

of Rac1 and inhibition of RhoA, intracellular kinases involved in cytoskeletal organization and contraction [118]. Further, Ang II activation can provoke a stabilized scaffold of integrin-related proteins such as FAK and Tln in cells, including ones in the cardiovascular system, producing more adherence to the ECM, thus influencing the fibrotic process [119].

Ang II also induces cardiovascular injury in part, by activating an inflammatory response. In two recent papers, macrophages and neutrophils have been shown to play an important role in cardiac fibrosis following Ang II infusion. In mice treated with Ang II, Tenascin-C was unregulated, accelerating macrophage migration and synthesis of proinflammatory and profibrotic cytokines in the myocardium, ultimately resulting in a significant fibrotic response [120]. Tenascin-C is a matricellular protein not detected in normal adult heart, but expressed in several heart diseases closely associated with inflammation. It binds to multiple cell surface receptors, including several integrins [121]. In one study, Shimojo et. al. demonstrated that αvβ3 is the functional receptor in macrophages for Tenascin-C, mediating migration and the inflammatory response [120]. In another study, Friedrichs et. al. demonstrated that Ang II-induced infiltration of neutrophils into atrial tissue was mediated by αMβ2 integrin. In turn, this caused fibrosis which promoted the initiation and propagation of atrial fibrillation [122].

Ang II is also a potent inducer of OPN expression in different cell types such as smooth muscle cells and fibroblasts [123]. OPN is a glycoprotein that acts as a cytokine and also as a matricellular protein when immobilized to the matrix. It is expressed by many immune cells such as macrophages, and is markedly upregulated in response to tissue injury [124]. OPN has two types of cell surface receptors, CD44 and integrins (αvβ1, αvβ3, αvβ5, αvβ6, α5β1, as well as α4 and α9 subunits) [125, 126], and is markedly upregulated after MI and in models of cardiac hypertrophy and fibrosis due to PO and Ang II infusion [127–129]. In a model of Ang II-induced fibrosis, OPN KO mice had markedly reduced fibrosis compare to WT mice [128]. OPN may thus promote matrix deposition by enhancing macrophage infiltration or by modulating fibroblast function. It has been shown to mediate proliferation in CFs stimulated with Ang II [130]. Whether interactions with specific integrins or CD44 mediate the profibrotic actions of OPN needs further investigation.

4.2 Integrins and integrin related proteins in TGFβ1 activation, Epithelial to Mesenchymal Transition (EMT) and fibrosis

TGFβ1 has been reported to be a key mediator of CF activation and is recognized as a major factor in the development of fibrosis in multiple organs and in a variety of cardiac pathologies [131]. In cardiac fibrosis and remodeling, TGFβ1 has been shown to be initially derived from immune cells and then subsequently to be produced by myofibroblasts [132]. Inhibition of TGFβ1 prevented late cardiac remodeling in a mouse MI model [133]. In PO induced cardiac remodeling, antibodies against TGFβ reduced myocardial fibrosis without affecting resultant hypertrophy or cardiac function [134]. TGFβ1 appears to be also implicated in the pathogenesis of age-related fibrotic cardiomyopathy that can potentially be a component of HFpEF. TGFβ1 heterozygous KO mice displayed an improved lifespan that was associated with reduced myocardial fibrosis and improved left ventricular compliance when mice were examined at 24 months of age [135].

There are three isoforms of TGFβ, and all are synthesized as precursor proteins that are processed by proteolytic cleavage in the endoplasmic reticulum and assemble as a non-covalent complex of a disulfide-linked homodimer of the mature cytokine (a short C-terminal fragment) and a disulfide-linked homodimer of a large amino terminal fragment termed the latency associated peptide (LAP) (Figure 2). The LAP homodimer prevents the mature TGFβ from binding to its receptors and therefore inhibits further downstream signaling from TGFβ. This complex termed the small latent complex, SLC, is further modified and linked to another family of proteins called latent TGFβ binding proteins (LTBP) to form the large latent complex (LLC). (Figure 2) Upon secretion LTBP is chemically cross-linked to the ECM, allowing it to store and tether TGFβ in a latent form in the extracellular space [136, 137]. Latent TGFβ1 is converted into its active form by various mechanisms. Proteolytic cleavage of LLC and liberation of active TGFβ1 can be performed by bone morphogenetic protein 1, several MMPs, plasmin, elastase, thrombin, and cathepsin. Independent of proteolytic cleavage, interaction between LAP and TSP-1 also promote latent TGFβ1 activation [138].

Integrins have also been shown to bind, and in some cases activate, latent TGFβ1. αv integrins (αvβ1, αvβ3, αvβ5, αvβ6, and αvβ8), integrins α5β1, α8β1 and the platelet integrin αIIbβ3, can bind latent TGFβ1 [139]. Integrins αvβ3, αvβ5, αvβ6 and αvβ8 have been shown to bind the RGD sequence in the LAP of TGFβ1 and TGFβ3 [140– 144].

Two main models are proposed to explain how integrins contribute to latent TGFβ1 activation. In the first, integrins αvβ8 and αvβ3 are suggested to simultaneously bind the latent TGFβ1 complex and MMPs. This allows the latent TGFβ1 complex and proteases to be positioned in close proximity and facilitates the enzymatic cleavage and release of active TGFβ1. Alternatively, it is suggested that integrins αvβ3, αvβ5, αvβ6 and αvβ8 change the conformation of the latent TGFβ1 complex by transmitting cell traction forces (Figure 2). This second mechanism requires the association of the latent complex with ECM, and is independent from proteolysis [138]. Knowing that different integrins use varied mechanisms to activate latent TGFβ depending on the cell type and organ, may help us to develop new therapeutic strategies directed specifically at the organ and cell type planned for the targeted therapy.

4.2.1 TGFβ1 activation by epithelial cells (αvβ6)—One of the best characterized integrin-dependent mechanisms of TGFβ activation has been studied in epithelial cells which contain the αvβ6 integrin receptor. Although this integrin is largely restricted to epithelial cells which are not expressed in heart, these studies provide a foundation to understand how integrins interface with TGFβ activation and thus are relevant to our discussion. Specifically, several studies support the role of αvβ6 integrin in TGFβ1 activation and link it with pulmonary, liver, kidney and skin fibrosis. In healthy lungs, the αvβ6 integrin is minimally expressed in alveolar epithelial cells. Yet after lung injury, it is rapidly upregulated [145]. Similarly, αvβ6 integrin expression is low in kidney and liver, but marked induction is observed in epithelial cells in several renal and hepatic diseases associated with inflammation and fibrosis [146–148]. Genetic ablation of β6 integrin expression or treatment with anti-αvβ6 antibodies or αvβ6 inhibitors (EMD527040) have

been shown to attenuate the accumulation of myofibroblasts and the interstitial deposition of collagen in models of lung, kidney and liver fibrosis [146, 148–150].

The αvβ6 integrin can bind directly to the LAP of TGFβ1 and TGFβ3. Cells expressing αvβ6 can activate TGFβ1 in vitro, a process which can be completely inhibited by anti-β6 integrin blocking antibodies [151]. Activation of TGFβ1 in these cells can also be inhibited by the use of actin polymerization and Rho inhibitors, which indicates the important role of force generation by the actin cytoskeleton in this process [142, 152]. This was confirmed by Shi and colleagues, in studies where they solved the crystal structure of the small latent complex of TGFβ and demonstrated that mechanical force generated by the contractile actomyosin cytoskeleton and transmitted by integrins, is a common mechanism to activate TGFβ [153]. (Figure 2)

In addition to integrins themselves, some integrin-related proteins have also been linked to TGFβ1 activation in epithelial cells and therefore in fibrosis. Depletion of kindlin-2 by siRNA ameliorated renal tubulointerstitial fibrosis after unilateral ureteral obstruction. In vitro studies showed that kindlin-2 knockdown in tubular epithelial cells suppressed the activation of TGFβ downstream signaling [154].

4.2.2 TGFβ1 activation by mesenchymal cells (fibroblasts, mesangial and hepatic stellate cells) (αvβ8, αvβ3, αvβ5)—Myofibroblasts are derived from mesenchymal cells and as discussed above, one of the main cell types involved in the fibrotic response in the myocardium. They are a major source of ECM proteins, and also can liberate and activate TGFβ1. Several studies have shown that integrins in mesenchymal cells are involved in the development of fibrosis in different organs and act via activation of TGFβ1. Though some of the following discussion centers on varied organs, the overall discussion has relevance to cardiac fibrosis, as parallel studies may evolve from future studies in heart.

αvβ8 integrin is increased in the airway fibroblasts of chronic obstructive pulmonary disease patients [155]. Conditional deletion of the β8 integrin subunit in fibroblasts inhibited airway fibrosis in IL-1β and ovalbumin–induced airway fibrosis, in mice [156]. Similar to αvβ6, αvβ8 also binds and activates TGFβ1, yet while TGFβ1 activation by αvβ6 is resistant to protease inhibitors, metalloprotease inhibitors abolish αvβ8-mediated TGFβ1 activation [141]. MT1-MMP (MMP14) has been shown to be the MMP involved in this process [141]. αvβ8 activates TGFβ1 by binding the RGD sequence in the LAP and presenting the latent complex to cell-surface MMPs, which in turn cleave and degrade LAP and then release the active TGFβ1 form [141].

In mesangial cells, modified pericytes in the kidney, αvβ8 appear to have the opposite effect. Its binding to the latent TGFβ1 complex maintained TGFβ1 in an inactive state [157]. This highlights how the same integrin can have different effects depending in the cell type and/ or organ in which it is expressed.

Hepatic stellate cells (HSC), the local pericyte population in the liver, are the major source of myofibroblasts in liver fibrosis. αvβ3 integrin is expressed in human and rat HSCs, and its inhibition reduced proliferation and increase apoptosis of these cells in vitro [158]. Though

still somewhat controversial, the use of cilengitide (a pentapeptide containing the RGD sequence and that acts as an inhibitor for αvβ3 and αvβ5 function) increased the collagen deposition in two models of liver fibrosis [159].

αvβ3 and αvβ5 are also found to be upregulated in skin fibroblasts from patients with scleroderma. Both of these integrins have been shown to be involved in activation of latent TGFβ1 in primary cultures of these cells. Further, treatment of these cells with antibodies against αvβ3 and αvβ5 reduced the expression of collagen I [140, 144, 160, 161].

As we discussed above, αv integrin-mediated activation of latent TGFβ1 is a key event which promotes fibrosis in many organs. In a paper by Henderson et al., αv integrin subunit expression was reduced in myofibroblasts, leading to marked reduction in fibrosis in liver, lung and kidney, identifying myofibroblast αv-containing integrins as components of a core pathway widely shared by pathological fibrosis in multiple organs [162]. In another recent paper from the same group [163], they developed a highly specific small molecule inhibitor of αvβ1 integrin (c8), which is highly expressed in active fibroblasts, and they obtained the same level of protection against fibrosis in liver and lung, as the one they reported in the mice with conditional deletion of all αv integrins from activated fibroblasts [162], suggesting that αvβ1 is the major αv integrin responsible for that effect.

Further, FAK expression and activity were upregulated in fibrotic foci in lungs from pulmonary fibrosis patients, while siRNA-mediated down-regulation or inhibition (PF-562,271) of FAK prevented bleomycin-induced lung fibrosis in mice. They also proved that FAK deficiency impaired myofibroblast differentiation [164]. FAK has been shown to be a key mediator of TGFβ signaling in lung and skin fibroblasts [165, 166].

The heart does not possess epithelial cells. Therefore, it is suggested that mesenchymal cells there may contribute to mechanical activation of latent TGFβ1. The mesenchymal integrins αvβ5 and αvβ3 are expressed in normal heart, and become upregulated during heart fibrosis [167] and after TGFβ1 and Ang II treatment of CFs [113]. Recent work identified integrins αvβ3 and αvβ5 as activators of latent TGFβ1 in human CF and showed their role in regulating CF to myofibroblast differentiation, through TGFβ1 activation [167]. Balasubramanian et. al., demonstrated that β3 integrin KO mice show a substantially reduced accumulation of interstitial fibronectin and collagen after PO, compared to control WT mice [88]. These results are compatible with the hypothesis that loss of β3 integrin expression, likely related specifically to αvβ3, reduced activation of TGFβ1. Proof of this hypothesis requires further investigation.

αvβ1 integrin is expressed in CFs but there is no information about how this integrin behaves in the heart undergoing remodeling, or in CFs stimulated with fibrogenic stimuli such as TGFβ1 or Ang II. Still, given recent data in other organs that implicates the important role of this integrin in organ fibrosis, work with relevant cardiac disease models should be explored to investigate the role of αvβ1 in cardiac fibrosis.

4.2.3 Integrins and the epithelial-mesenchymal transition (EMT)—As mentioned above, the origin of myofibroblasts has been intensely studied, with potential sources

including resident fibroblasts, circulating progenitors, and also the possible derivation during the process of epithelial – mesenchymal transition (EMT) [10, 168]. EMT involves the conversion of differentiated epithelial cells into fibroblasts and myofibroblasts. This process occurs normally during embryonic development, but in aberrant wound repair processes, EMT may also lead to fibrosis. EMT has been associated with fibrosis in several organs such as kidney and lung. TGFβ1 is one of the main factors triggering EMT.

In lung, this process has shown to be regulated by the epithelial integrin α3β1. Mice with epithelial cell specific deletion of α3 integrin showed a reduction in myofibroblast number and collagen I deposition in the lungs, following bleomycin injury (a known inducer of fibrosis) compared to control treated mice [169]. In addition, the integrin related proteins ILK, PINCH and FAK have shown to be involved in EMT and kidney fibrosis [170–173]. Although earlier reports supported the contribution of epithelial cells to the myofibroblast population in kidney fibrosis, more recent genetic tracing studies failed to confirm these observations [174, 175]. Still, inhibition of EMT has been shown to be protective against kidney and lung fibrosis. Recent studies suggest that the pro-fibrogenic consequences of EMT activation are not mediated by simply inducing myofibroblast proliferation, but rather also occurs through impaired regenerative potential and cell-cell communication of epithelial cells [176, 177].

Endothelial–mesenchymal transition (EndMT) is a complex biological process distinct from, but similar to EMT, whereby ECs lose their specific markers and acquire a mesenchymal or myofibroblastic phenotype. With this they express mesenchymal cell products such as α-SMA and type I collagen. EndMT was originally observed during heart development, but recent studies have suggested that it also plays a role in pathological settings such as cancer and fibrosis. Several papers have shown that cardiac fibrosis is associated with the emergence of fibroblasts originating from ECs, and that TGFβ1 is the main factor that induces this process, suggesting that EndMT may well be relevant not only in development, but also in cardiac fibrotic disease [178–180]. Unfortunately, no studies about the regulation of this process by integrins or integrin related proteins in cardiac fibrosis has been performed to date. Still recent papers using genetic tracing techniques demonstrate that the contribution of EndMT to the fibroblast/ myofibroblast population after TAC and MI is negligible [181, 182], yet the authors did not rule out the possibility that this process could contribute to cardiac fibrosis without contributing to the myofibroblast population, as happens in kidney fibrosis [176, 177].

4.3 Integrins in mechanical stress and cardiac fibrosis

Beside biochemical factors, such as Ang II and TGFβ1 mentioned above, cues produced via mechanical strain and ECM stiffness, also play important roles in regulating myofibroblast differentiation and therefore fibrosis. Specifically, lung fibroblasts cultured on a stiff matrix have higher rates of proliferation and α-SMA expression, but decreased apoptosis compared with those grown on a compliant matrix [183]. Similarly, hepatic stellate cells become progressively activated with increasing levels of mechanical tension in the ECM [184]. On the other hand, decreasing substrate stiffness on which myofibroblasts are cultured, has been

shown to reverse myofibroblast differentiation and promote the quiescent fibroblast phenotype, in vitro [185].

Cardiac cells reside within one of the most mechanically dynamic environments of the body, and with each contraction they experience cyclic strain (relative deformation) and stress (force per unit area). During pathological conditions such as occur with MI or PO, those parameters change, affecting cardiac cell behavior. Several studies show that CFs respond in a varied way to stimulation by different magnitudes of strain, particularly as regards differentiation of myofibroblasts [186]. Furthermore, as mentioned above for other types of organs, several studies have shown that myofibroblast differentiation from CFs is regulated by ECM stiffness [187, 188]. Indeed, the relative high stiffness of injured and fibrotic areas within the heart may promote myofibroblast differentiation, which could prolong the existence of fibrosis via increase secretion of TGFβ [189].

Integrins are mechanosensor adhesion proteins that transduce mechanical signals between the cells and their microenvironment, stimulating cellular responses such as cell growth and differentiation. Early studies in CFs by MacKenna et al demonstrated that integrins act as mechanotransducers in these cells, activating downstream signaling in response to mechanical stretch [190]. As mentioned above, β1 integrins regulate the angiotensinogen gene in CFs, through p38 MAPK signaling, in response to mechanical stretch [118]. This effect is mediated by Rac1 and RhoA kinases. These two cases are examples of integrin outside-in signaling. In addition, as also mentioned above, integrins can participate in inside-out signaling. In this case, intracellular forces generated by actin fibers, can be transmitted through integrins to the ECM to alter its organization. A recent paper demonstrates that β1 integrin binding plays a role in the constant traction force generation in response to varying stiffness for cells grown on cardiac ECM [191]. One relevant integrin inside-out signaling case relevant for fibrosis, is the activation of TGFβ1. TGFβ1 is secreted into the ECM in an inactive form, mechanical force generated by the contractile actomyosin cytoskeleton and transmitted by αv integrins, can change the conformation of the latent TGFβ1 complex, releasing its active form [153]. The contribution of mechanobiology and mechanosensing to the development of fibrosis are just beginning to emerge and their role in the myocardium requires significantly more study.

5. Integrins and FAK as therapeutics targets against fibrosis. Could manipulation of integrin expression / function be used therapeutically to modify cardiac fibrosis?

Because TGFβ has been shown to have a crucial role in promoting the fibrotic response, initial approaches to fight fibrosis in different organs have focused on inhibition of this cytokine. Many of these studies have been hampered due to the multifunctional nature of TGFβ. Due to the anti-proliferative role of TGFβ on epithelial cells, its inhibition could have carcinogenic effects, which is particularly relevant for liver fibrosis [192]. Due to the critical role of TGFβ as an immunosuppressant, generalized blockade of TGFβ activity may lead to excessive autoimmunity and inflammation [193, 194]. Therefore, some anti-fibrotic strategies that aim to diminish TGFβ signaling or prevent TGFβ activation have gained much

more relevance in the fight against fibrosis. As above, the latent TGFβ complex may be activated by proteolytic mechanisms or by traction forces mediated by integrins (mainly αvβ6, αvβ3 and αvβ5). In this regard, an αvβ6-specific monoclonal antibody is undergoing phase II evaluation to treat idiopathic pulmonary fibrosis [195]. Small molecules inhibitors such as EMD527040, a non-peptide αvβ6 integrin antagonist, have also shown to have therapeutic potential in animal models of liver fibrosis [150]. Also, a small molecule inhibitor for all αv integrins (CWHM12), has been shown to be effective in the prevention of liver and lung fibrosis in a mouse model [162]. Recently, a small molecule (c8) that inhibits αvβ1 has been also found to be very effective against pulmonary and liver fibrosis [163]. Therefore, these two compounds could have a great potential to be broadly useful for treatment of diseases associated with excessive tissue fibrosis. Future studies will show the relevance of this work in other organs to treatment of cardiac fibrosis.

Although the myocardium does not have epithelial cells, inhibition of mesenchymal integrins in CFs (αvβ3, αvβ5, and αvβ1), could be potential targets to suppress cardiac fibrosis through the inhibition of TGFβ activation and fibroblast - myofibroblast differentiation. Therefore, the use of small molecules inhibitors such as CWHM12 or c8, should be evaluated in models of cardiac fibrosis. Still, one must be aware that generalized inhibition of αvβ3 and αvβ5 integrins could affect function of these integrins in ECs, thus altering angiogenesis that can be required for appropriate post-MI remodeling. Another important issue that has to be considered in post-MI remodeling is the timing of the treatment administration after MI. As it is broadly accepted, treatments that decrease collagen production should be avoided early after MI because they could jeopardize the formation of the mature scar and lead to ventricular rupture, therefore we posit that use of integrin inhibitors that target TGFβ activation should be avoided during early phases of remodeling when they could affect collagen deposition and scar formation.

A variety of in vitro and in vivo studies with FAK inhibitors, suggests that FAK plays a key role in the development of fibrotic disorders in lung, skin and liver, and thus it appears to be an attractive target for anti-fibrotic therapy [196]. In recent years, several orally bioavailable ATP-competitive FAK inhibitors have been developed and have entered in clinical trials as anti-tumor treatments [197, 198]. PF-562,271 was one of the first clinically available FAK inhibitors. It inhibits FAK phosphorylation and in studies with animals has been shown to prevent bleomycin-induce lung fibrosis [164]. Another, FAK inhibitor PF-573,228 has been shown to be effective against skin fibrosis [199]. To date, no clinical studies in humans have evaluated the effects of FAK inhibitors in fibrotic diseases.

In the heart, FAK has been associated with CF proliferation, migration, in response to growth factors such as PDGF [84]. FAK has also been associated with cardiac fibrosis in in vivo models of MI and PO [87, 200]. Therefore, FAK inhibitors could be another potential therapeutic tool against cardiac fibrosis, although, as before, the timing of their use in MI patients may be difficult to predict, as importantly they should be limited to use in later phases of remodeling, when the scar is completely mature. Further studies in animal models of cardiac fibrosis with FAK inhibitors will be necessary to evaluate the potential use of these treatments.

6. Conclusion and future directions

A great amount of effort has been focused on the study of the varied mechanisms and factors that regulate fibrosis in different organs. Integrins have been shown to be one of the main common regulatory factors of this process. (Figure 3) They are able to regulate the main cytokine involved in myofibroblast trans-differentiation, TGFβ1, and are also well known as transducers of mechanical stress. It is apparent that increased integrin expression in specific cell types, may be linked to fibrosis. This has made them an attractive anti-fibrotic therapeutic target. Recently, several integrin inhibitors have been developed and preliminary animal studies have shown great potential for their use against fibrosis in multiple organs.

The study of integrins in cardiac fibrosis has not been as extensively investigated as it has in other organs. Indeed, some of the cardiac-oriented studies have simply been descriptive or observational in nature. Additional studies using genetically modified animals (knockouts, transgenics, etc.) with specific attention to sub-types of cardiac cells evaluated, as well as studies with specific integrin inhibitors, are necessary to better understand the role of integrins in the development of cardiac fibrosis. Since development of cardiac fibrosis is a factor in many cardiac pathologies that can lead to frank heart failure, development of new treatments focused on modulating integrin and integrin-related protein function may offer novel treatments that can favorably alter the cardiac remodeling process. Much new work is required for this purpose.

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Abbreviations

α-SMA	α-smooth muscle actin
Ang II	Angiotensin II
CFs	Cardiac fibroblasts
CM	Cardiac myocyte
ED-A FN	ED-A fibronectin
EC	Endothelial cells
EndMT	Endothelial–mesenchymal transition
EMT	Epithelial – mesenchymal transition
ECM	Extracellular matrix
FAK	Focal adhesion kinase
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HSC	Hepatic stellate cells

ILK	Integrin-linked kinase
LLC	Large Latent Complex
LAP	Latency Associated Peptide
LTBP	Latent TGFβ Binding Proteins
LV	Left Ventricle
LFA-1	Lymphocyte function-associated antigen
Mac-1	Macrophage-1 antigen
MMP	Metalloproteinase
MI	Myocardial infarction
OPN	Osteopontin
Pax	Paxillin
PECAM-1	Platelet Endothelial Cell Adhesion Molecule
PDGF	Platelet-derived growth factor
PO	Pressure overload
Pyk2	Proline-rich tyrosine kinase-2
SIV	Simian immunodeficiency virus
SLC	Small latent complex
STZ	Streptozotocin
VSMC	Vascular smooth muscle cells
Tln	Talin
TSP-1	Thrombospondin-1
TGFβ1	Transforming growth factor beta 1
Vcl	Vinculin

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Highlights

- Cardiac fibrosis is one of the major components of pathological cardiac remodeling
- Integrins regulate key effectors that drive fibrosis in many organs including heart
- Integrins are potential therapeutic targets to treat cardiac fibrosis

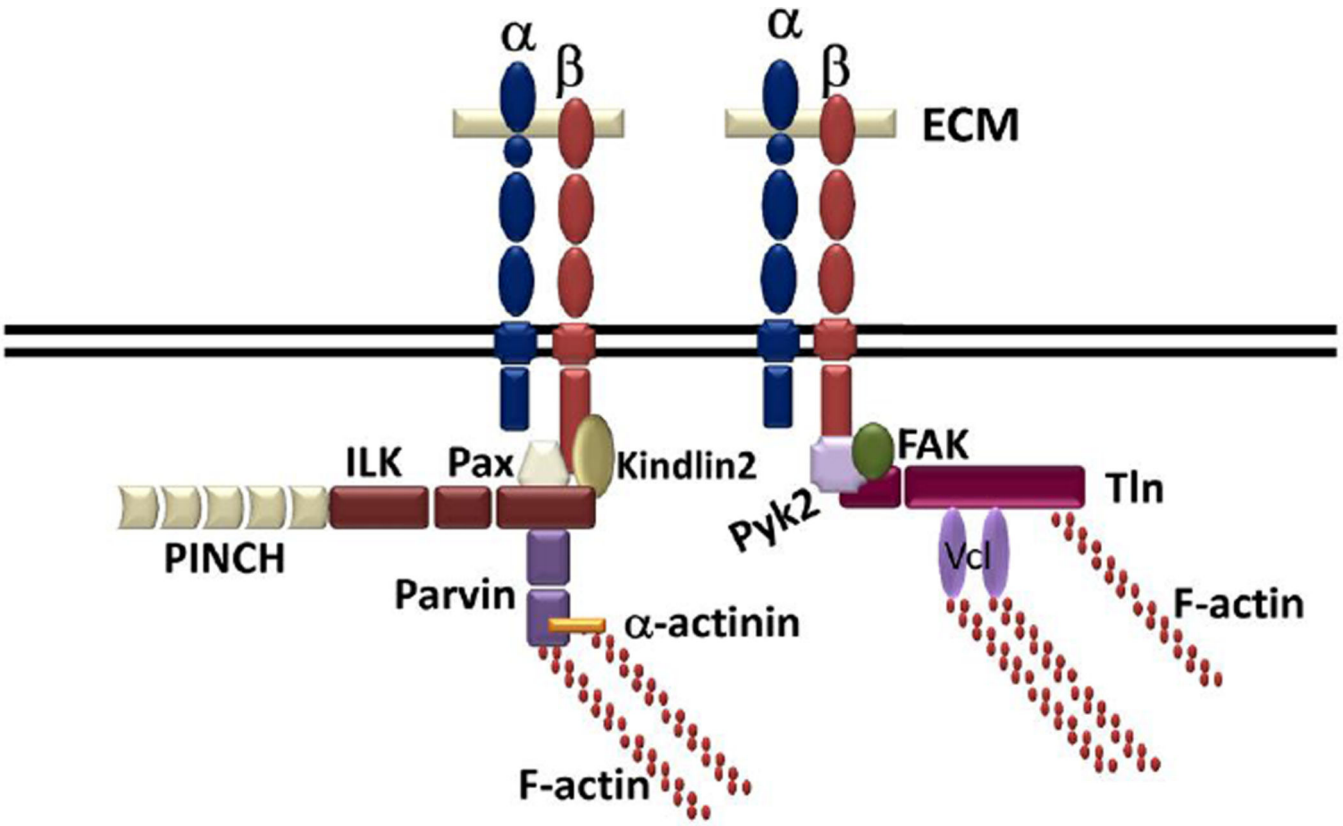

Figure 1. Integrins and Integrin-related proteins

Integrins shown spanning the cell membrane, connect and aggregate a range of adapter and signaling proteins such as integrin linked kinase (ILK), focal adhesion kinase (FAK), paxillin (Pax), vinculin (Vcl), talin (Tln), Kindlin, PINCH, Parvin, actinin and even actin. This allows both bridging of ECM to the intracellular cytoskeleton, and also allows propagation of signals bidirectionally across the cell membrane.

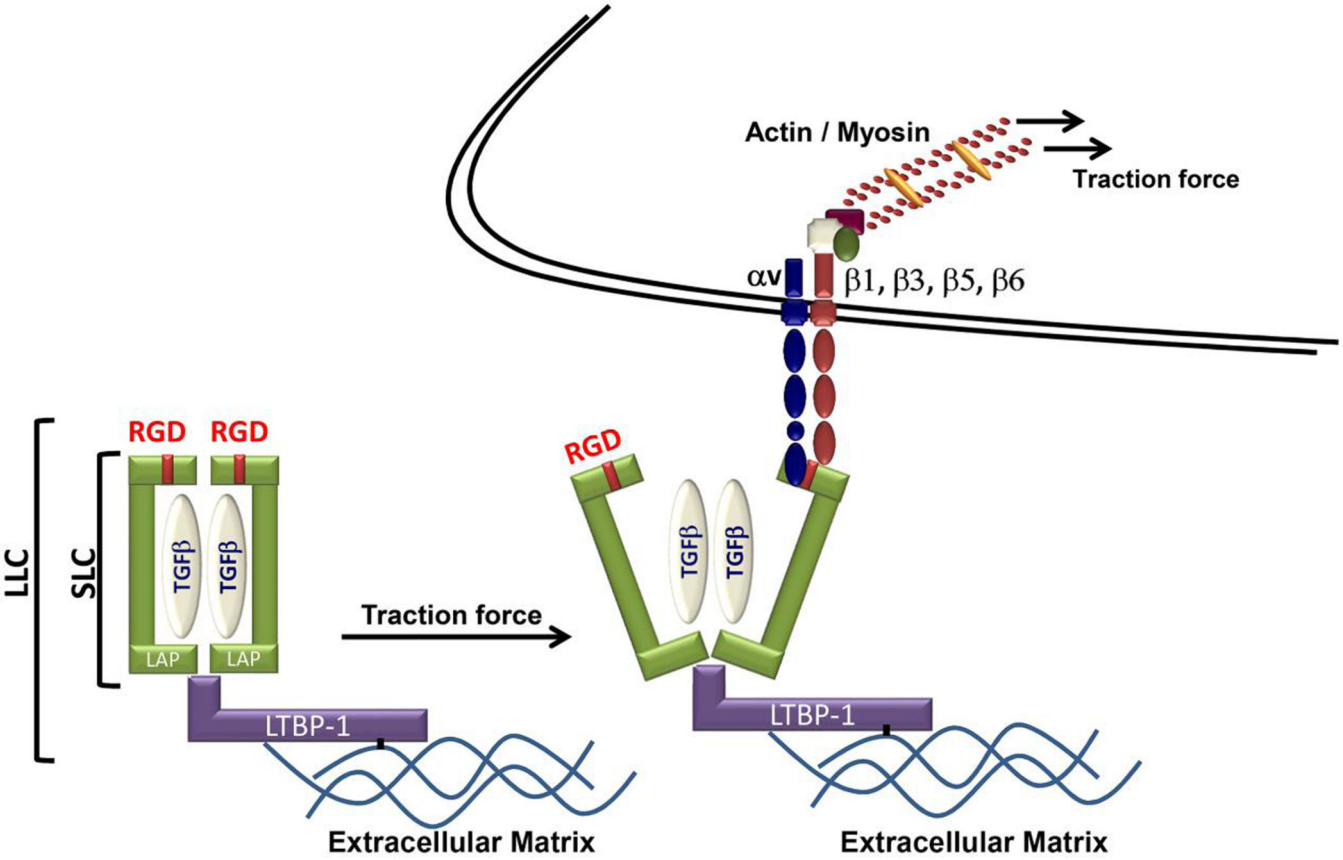

Figure 2. Model of mechanical activation of latent TGFβ1

TGFβ1 is secreted in a large latent complex (**LLC**) that consists of TGFβ1 associated with LAP (Latency Associated Peptide), the short latent complex (**SLC**), and LTBP-1 (Latent TGFβ1 Binding Protein 1). LAP contains the amino acid sequence motif RGD (Arg-Gly-Asp) which serves as a recognition site for several integrins. Actin / myosin-mediated cell contraction force can be transmitted to an RGD binding site in LAP through the αv integrins and induces a putative conformational change that liberates TGFβ1, activating it.

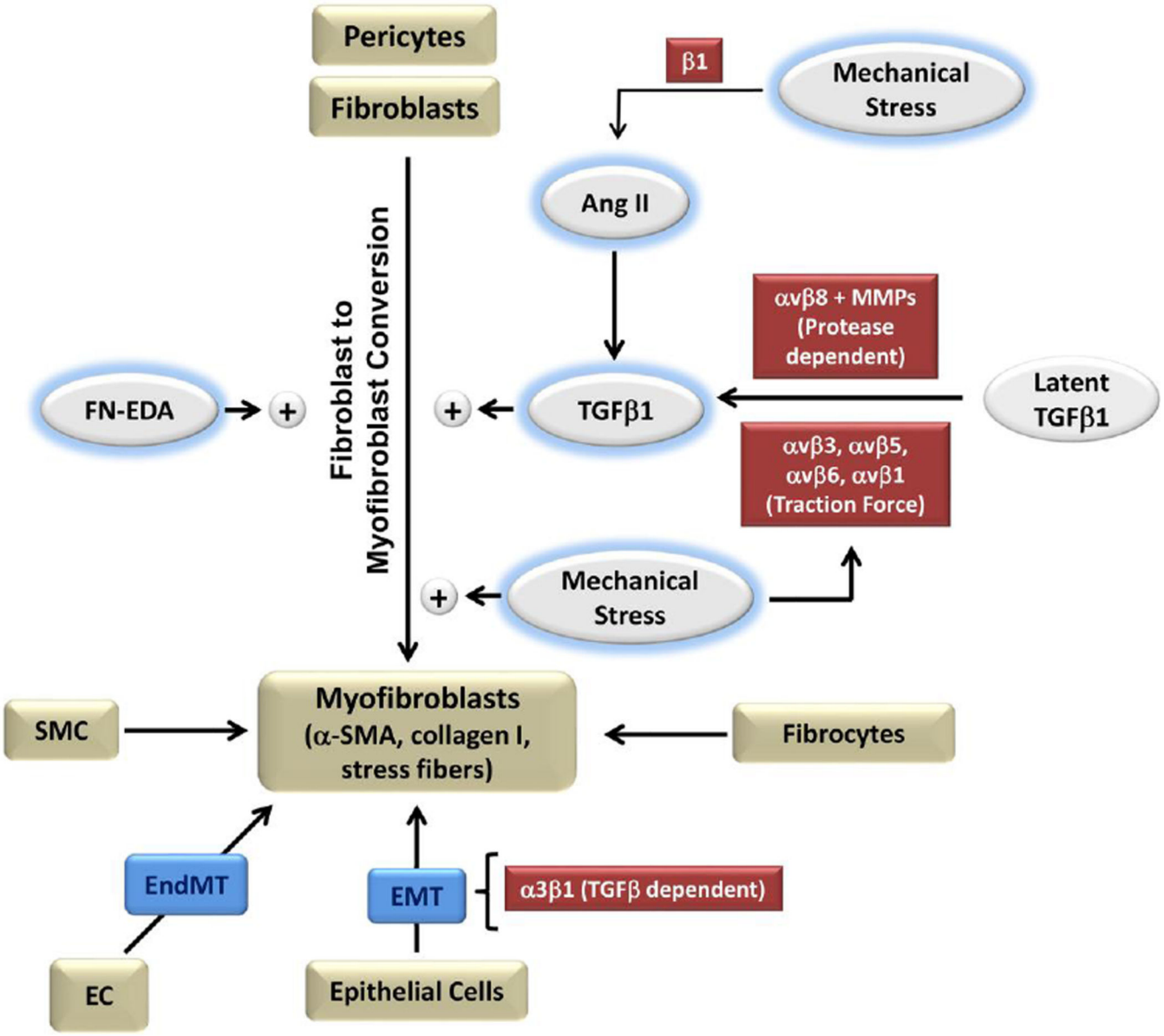

Figure 3. Myofibroblast precursors and integrin subtypes involved in end-effector regulation

Myofibroblasts can be derived from resident fibroblasts and pericytes, as well as from smooth muscle cells (SMC), endothelial cells (EC), epithelial cells and fibrocytes. As one example, in response to angiotensin II (Ang II), transforming growth factor beta-1 (TGFβ1), can increase the conversion of fibroblasts to myofibroblasts. Mechanical stress (perhaps functioning through Ang II) and ED-A fibronectin (ED-A FN) can also increase this transformation. Integrins are thus involved in TGFβ activation, in EMT regulation and in Ang II synthesis. Myofibroblasts are characterized by the presence of α-smooth muscle actin (α-SMA), stress fibers and collagen production. EMT (epithelial - mesenchymal transition); EndMT (endothelial - mesenchymal transition). + denotes factors can positively increase the transformation of fibroblasts to myofibroblasts.